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European Neuropsychopharmacology 13 (2003) 228–234

EUROPEAN NEURO-PSYCHOPHARMACOLOGY

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Distribution of 5-HT_4 receptors in the postmortem human brain—an autoradiographic study using [^{125}I]SB 207710

Katarina Varnäs[a], Christer Halldin[a], Victor W. Pike[b], Håkan Hall[a,*]

[a]*Karolinska Institutet, Department of Clinical Neuroscience, Psychiatry Section, Karolinska Hospital, S-17176 Stockholm, Sweden*
[b]*Molecular Imaging Branch, National Institute of Mental Health, Building 1, Room B3-10, 1 Center Drive, Bethesda, MD 20850-0135, USA*

Received 29 January 2002; received in revised form 27 June 2002; accepted 17 September 2002

Abstract

The autoradiographic distribution of the 5-HT_4 receptor was described using human postmortem brain sections and the selective radioligand [^{125}I]SB 207710 [(1-*n*-butyl-4-piperidinyl)methyl-8-amino-7-[^{125}I]iodo-1,4-benzodioxane-5-carboxylate]. The specific binding was highest in regions of the basal ganglia (caudate nucleus, putamen, nucleus accumbens, globus pallidus and substantia nigra) and the hippocampal formation (CA1 and subiculum). In the neocortex, the binding showed a distinct lamination pattern with high levels in superficial layers and a band displaying lower levels in deep cortical layers. The results confirm previous studies on the distribution of 5-HT_4 receptors in the human brain in vitro and provide high-resolution correlates for in vivo imaging studies using the radioligand recently developed for single photon emission tomography (SPET), [^{123}I]SB 207710.

Keywords: [^{125}I]SB 207710; 5-HT_4 receptors; Human brain; Whole hemisphere autoradiography

1. Introduction

The 5-HT_4 receptor has been identified in various tissues as a G-protein coupled receptor positively linked to adenylyl cyclase activity (Hoyer et al., 1994). The presence of 5-HT_4 receptors has been described in the brain of several species (Domenech et al., 1994; Jakeman et al., 1994), including human (Bonaventure et al., 2000; Domenech et al., 1994; Mengod et al., 1996; Reynolds et al., 1995; Waeber et al., 1993). High densities of 5-HT_4 receptors are found in regions of the basal ganglia with lower levels in the hippocampal formation and the neocortex (Bonaventure et al., 2000; Domenech et al., 1994; Grossman et al., 1993; Jakeman et al., 1994; Reynolds et al., 1995; Waeber et al., 1993, 1994). In the human brain, 5-HT_4 receptor mRNA levels are high in the hippocampal formation and in the striatum but are, in contrast to the receptor proteins, absent in the globus pallidus and the substantia nigra, indicating that 5-HT_4 receptors are localised in terminals of striatal projections to these regions (Bonaventure et al., 2000).

While 5-HT_4 receptors in the alimentary tract are suggested to be important targets for the treatment of gastrointestinal motility disorders (De Ponti and Tonini, 2001), the function of central 5-HT_4 receptors is less well established. However, the distribution pattern in combination with neurochemical and behavioural observations indicates that brain 5-HT_4 receptors may be involved in cognition and anxiety (Eglen et al., 1995).

There are a few reports comparing brain 5-HT_4 receptor densities postmortem in subjects with neuropsychiatric disorders with those of normal control subjects. Reynolds et al. (1995) observed lower levels of 5-HT_4 receptors in the putamen of brains of patients affected by Huntington's disease and also reduced 5-HT_4 receptor densities in the hippocampus and frontal cortex in Alzheimer's disease patients compared to controls. On the other hand, no differences were found in the brains from parkinsonian patients (Reynolds et al., 1995). Furthermore, no differences in 5-HT_4 receptor densities could be detected in the

*Corresponding author. Tel.: +46-8-5177-4792; fax: +46-8-5177-1717.
E-mail address: hakan.hall@ks.se (H. Hall).

0924-977X/03/$ – see front matter
doi:10.1016/S0924-977X(03)00009-9

dorsolateral prefrontal cortex of brains from schizophrenic patients compared to controls (Dean et al., 1999).

In the present study, we have used the 5-HT_4 receptor antagonist radioligand [^{125}I]SB 207710 [(1-*n*-butyl-4-piperidinyl) methyl-8-amino-7-[^{125}I]iodo-1,4-benzodioxane-5-carboxylate] (Brown et al., 1993) to examine the distribution of 5-HT_4 receptors in the postmortem human brain by autoradiography. Binding studies indicate that this compound shows high affinity for 5-HT_4 receptors (K_D values of 86 and 37 pM in piglet hippocampus and caudate membranes, respectively) and about 1000-fold selectivity compared to other 5-HT receptors (Brown et al., 1993). [^{125}I]SB 207710 has also been used in autoradiographic studies to visualise brain 5-HT_4 receptors in rat (Patel et al., 1995; Vilaro et al., 1996) and several other species, including human (Mengod et al., 1996).

Iodine-123 labelled SB 207710 has recently been shown to be an effective radioligand for single photon emission tomography (SPET) studies in vivo (Pike et al., 1999). Thus, in a SPET investigation of the cynomolgus monkey brain, [^{123}I]SB 207710 showed suitable in vivo characteristics and was accumulated in 5-HT_4 receptor-rich regions including the striatum and the neocortex (Pike et al., 1999). This preliminary finding reinforces further characterisation of SB 207710 as radioligand for the study of 5-HT_4 receptors in the human brain in vivo. In the present study, we used human postmortem brain whole hemisphere autoradiography (Hall et al., 1998, 2001) to provide detailed high resolution anatomical correlates for the lower resolution in vivo studies.

2. Experimental procedures

2.1. Compounds

[^{125}I]SB 207710 was prepared from the precursor SB 207715 [(1-*n*-butyl-4-piperidinyl)methyl-8-amino-7-tributylstannyl-1,4-benzodioxane-5-carboxylate]. SB 207715 was incubated in [^{125}I]sodium iodide solution (no-carrier-added) in the presence of chloramine-T. The radioligand (specific radioactivity, 2200 Ci/mmol) was purified on a reverse phase column (μ-Bondapak C18), evaporated and dissolved in 75% ethanol. The radiochemical yield was 60% and the radiochemical purity was over 99%. Formulated [^{125}I]SB 207710 was radioactively stable during the period of investigation. Other compounds and chemicals were obtained from commercially available sources and were of analytical grade wherever possible.

2.2. Brain tissue

Human brains were obtained postmortem by clinical autopsy at the National Institute of Forensic Medicine, Karolinska Institutet, Stockholm, Sweden. The study was approved by the Ethics Committee at Karolinska Institutet and the Swedish Board of Social Welfare. Whole hemispheres (see Table 1 for details) were removed, frozen and cryosectioned as described earlier (Hall et al., 1998, 2001), using a heavy-duty cryomicrotome (Leica Cryomacrocut CM3600, Leica, Nussloch, Germany). The tissue cryosections (thickness 100 μm) were transferred to gelatinised or poly-L-lysine-treated glass plates (10×22 cm), dried at room temperature and then stored with dehydrating agents (−25 °C) until use.

2.3. Autoradiography

The autoradiographic experiments were carried out essentially as described previously (Hall et al., 1998, 2001). The sections were incubated for 60 min at room temperature with [^{125}I]SB 207710 (ca. 17 pM) in Tris–HCl buffer (pH 7.4, 50 mM) containing $MgCl_2$ (5 mM), EGTA (1 mM) and pargyline (10 μM). The non-selective agonist, serotonin (10 μM), was included in the incubation medium for anatomically adjacent sections to determine the level of non-specific binding. After incubation, sections were washed with cold Tris buffer (pH 7.4, 50 mM) containing $MgCl_2$ (5 mM) and EGTA (1 mM) for 3×10 min, briefly dipped into distilled water and dried on a warm plate. Hyperfilm βmax or Kodak BioMax MR films (Amersham Pharmacia Biotech, Uppsala, Sweden) were applied to the sections for 4 days before development (developer: Kodak D19; fixation: Kodak Fixer 3000).

Autoradiograms were digitised using a Scan Maker E6 high-resolution scanner (Microtek) and Adobe Photoshop 5.0. Microsoft PowerPoint was used for processing of the images. Measurements were carried out using Adobe

Table 1
Demographic data and description of brains used

No.	Age (years)	Hemisphere	Gender	Postmortem time (h)	Cause of death
46	63	Left	Male	48	Myocardial infarction
60	46	Left	Male	42.9	Accidentally shot during hunting
62	53	Right	Male	15.3	Myocardial infarction
70	61	Left	Male	15.4	Heart failure
71	55	Right	Male	29	Myocardial infarction
73	58	Left	Female	6	Myocardial infarction

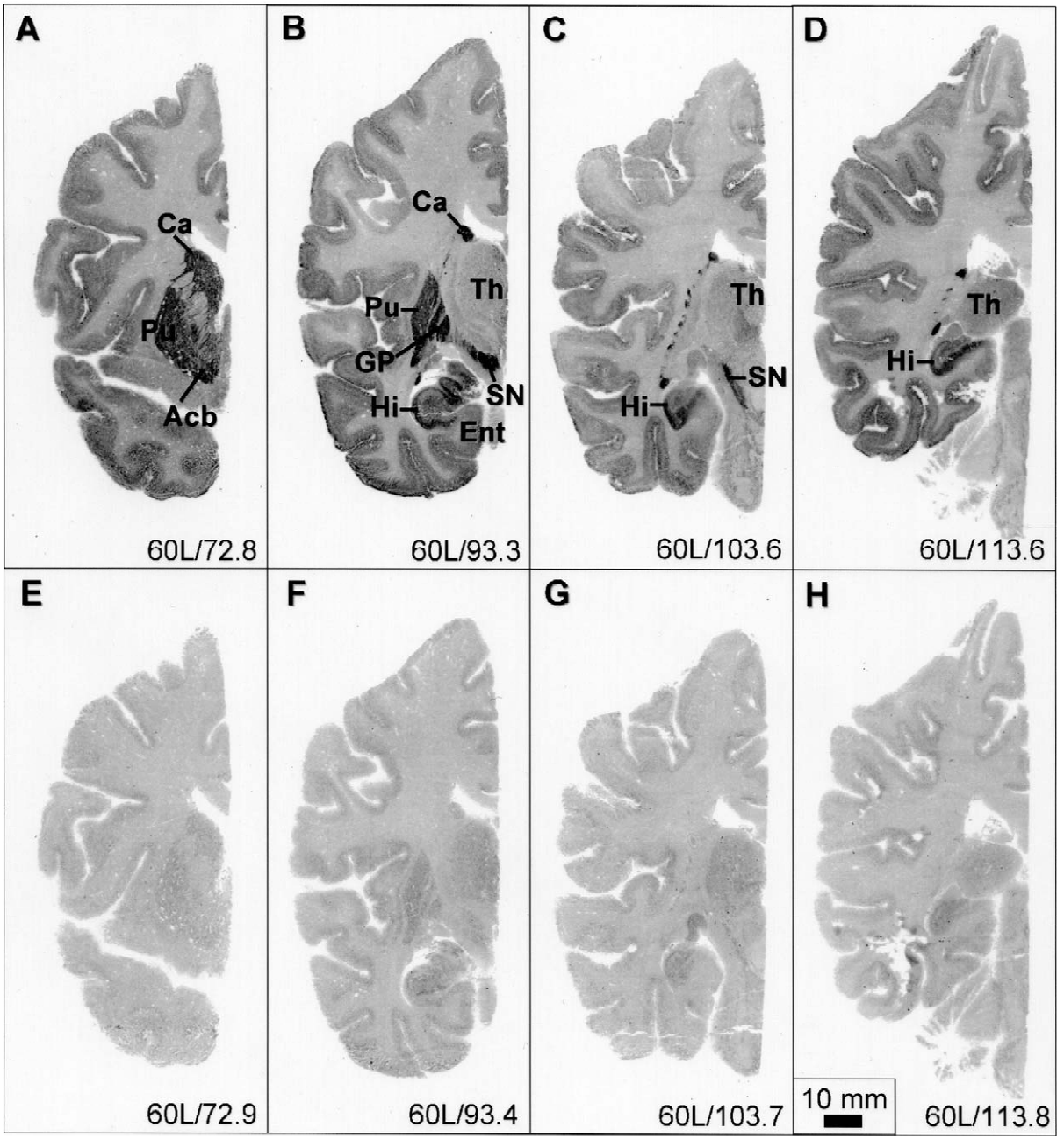

Fig. 1. Whole hemisphere autoradiograms showing the distribution of [^{125}I]SB 207710 binding to coronal sections from four levels of the human brain. (A–D) Total binding. (E–H) Non-specific binding, in the presence of serotonin (10 μM). General figure information: Numbers in lower right corner represent internal brain number and distance (in mm) from the frontal pole (coronal sections, brain 60L) or from the vertex (horizontal sections, brains 46L, 71R and 73L). Abbreviations: Acb, nucleus accumbens; Amg, amygdala; Ca, caudate nucleus; CA1 and CA3, Ammon's horn of the hippocampus; Cer, cerebellum; Cl, claustrum; DG, dentate gyrus; Ent, entorhinal cortex; GP, globus pallidus; Hi, hippocampus; Ins, insular cortex; mOcx, medial occipital cortex; pCG, posterior cingulate gyrus; PHG, parahippocampal gyrus; Pu, putamen; S, subiculum; SN, substantia nigra; Th, thalamus.

Photoshop 6.0. To quantify the density of [^{125}I]SB 207710 binding, pixel values obtained were transformed into radioactivity values (pCi/mm^2) using ^{125}I-calibrating scales (Microscales, Amersham, UK). The specific levels were calculated by subtracting non-specific levels from the total [^{125}I]SB 207710 radioactivity levels.

3. Results

3.1. General

The general brain distribution of [^{125}I]SB 207710 is shown in the autoradiograms obtained using coronal (Fig.

1) and horizontal (Fig. 2) sections. High levels of [^{125}I]SB 207710 binding sites were observed in regions of the basal ganglia and the hippocampus with lower levels in the neocortex. Generally, the non-specific binding of [^{125}I]SB 207710, as determined in the presence of 10 μM serotonin, was relatively low. Accumulation of radioactivity in the white matter was relatively high and was to some extent blocked by serotonin (Table 2).

3.2. Basal ganglia

The specific binding of [^{125}I]SB 207710 was similarly high throughout the basal ganglia (caudate nucleus, putamen, nucleus accumbens, globus pallidus and the substantia nigra, Figs. 1 and 2). The binding was heterogeneous in the caudate-putamen, with a pattern of low-density patches that was most evident in the putamen (Fig. 3A).

3.3. Neocortex

The neocortical binding pattern was similar in all regions with high levels in superficial layers (probably corresponding to layers I–II) and lower in deep cortical layers close to the white matter (Fig. 3B–D). The medial occipital cortex, particularly internal layers, was less densely labelled compared to other cortical regions analysed (Fig. 3C, Table 2). The specific binding seemed to be higher in the temporal cortex than in other regions of the neocortex (Table 2).

3.4. Limbic cortices

In the hippocampal formation, high levels of specific [^{125}I]SB 207710 binding were observed in the CA1 region and the subiculum with lower levels in the CA3–4 and the dentate gyrus. The binding was very low in the parahip-

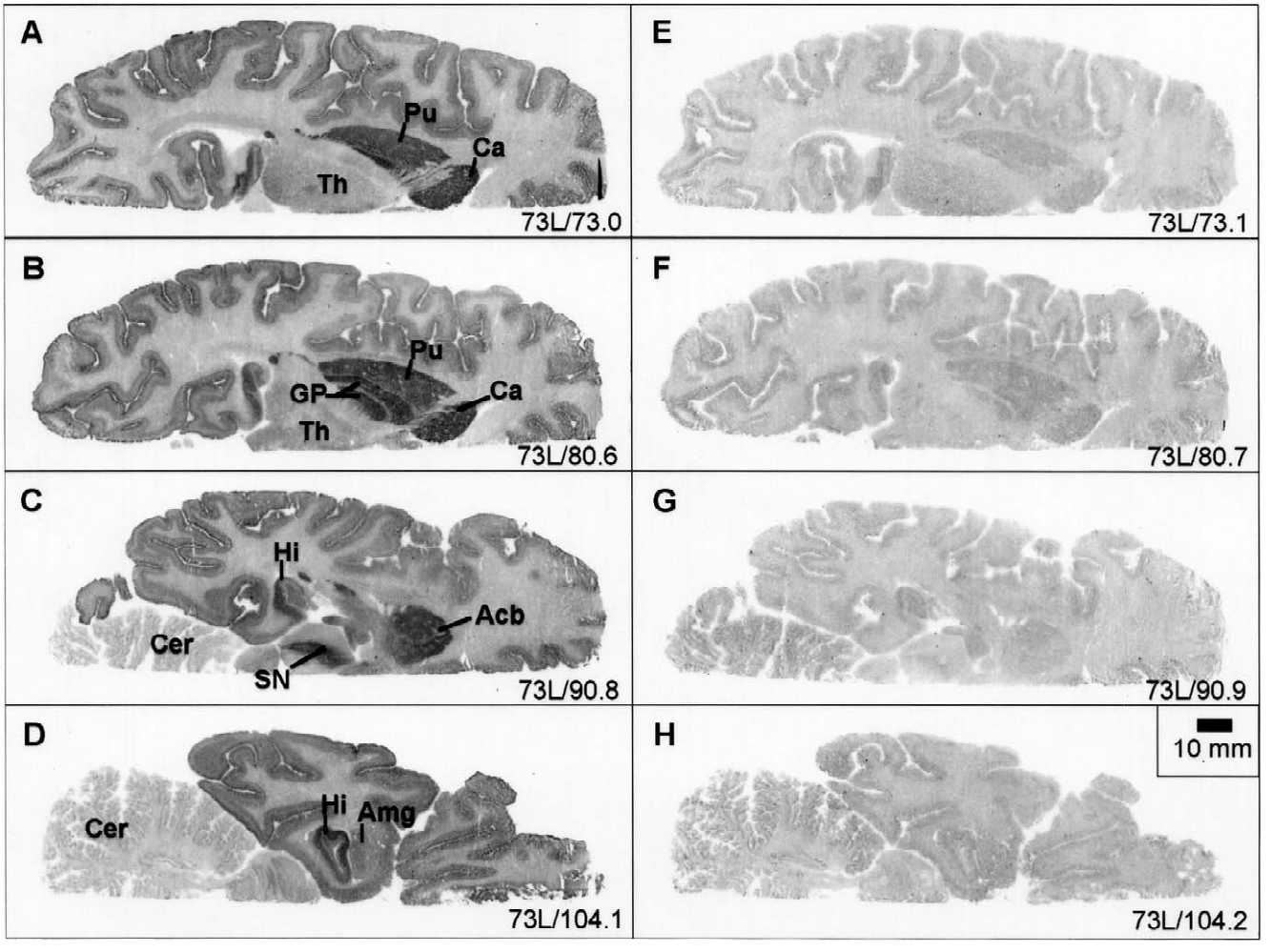

Fig. 2. Whole hemisphere autoradiograms showing the distribution of [^{125}I]SB 207710 binding to horizontal sections from four levels of the human brain. (A–D) Total binding. (E–H) Non-specific binding, in the presence of serotonin (10 μM). Abbreviations, see Fig. 1 legend.

Table 2
Autoradiographic distribution of [^{125}I]SB 207710 binding sites in the postmortem human brain

Brain region	*n*	Mean	S.D.
White matter	20	4.3	2.1
Neocortex			
Frontal cortex (inferior gyrus), external layers	13	18	6.2
Frontal cortex (inferior gyrus), internal layers	13	12	3.5
Insular cortex, external layers	12	19	3.9
Insular cortex, internal layers	12	13	2.4
Temporal cortex (medial gyrus), external layers	19	26	7.2
Temporal cortex (medial gyrus), internal layers	19	18	4.5
Occipital cortex, external layers	8	16	5.7
Occipital cortex, internal layers	8	7.8	5.9
Hippocampal formation			
CA1 field	14	30	11
Dentate gyrus	4	17	5.2
Amygdala (basolateral complex)	5	18	8.5
Basal ganglia			
Caudate nucleus	9	32	5.3
Putamen	11	26	2.8
Nucleus accumbens	5	29	2.4
External globus pallidus	5	31	4.4
Internal globus pallidus	2	30	
Substantia nigra	5	30	5.1
Thalamus	7	6.2	3.1
Anterior nuclei	5	13	3.6
Pulvinar nuclei	4	9.1	2.4
Lateral geniculate nucleus	2	1.2	
Brainstem			
Raphe nuclei	2	11	
Pontine nuclei	3	10	0.81
Cerebellum	6	0.35	0.40

Values are presented as specific binding and are expressed in pCi/mm^2.

pocampal gyrus (Fig. 3D). Low densities were detected in the amygdala (Fig. 3D, Table 2).

3.5. Thalamus

Low, but non-negligible specific binding was detected in the thalamus, with the highest levels in the anterior nuclei and slightly lower in the pulvinar nucleus (Table 2). Binding in the lateral geniculate nucleus was only to a minor extent blocked by excess serotonin, assuming very high levels of non-specific binding in this region.

3.6. Brainstem and cerebellum

Low levels of [^{125}I]SB 207710 binding were observed in the raphe and pontine nuclei (11 and 10 pCi/mm^2, respectively).

No evidence for specific binding to 5-HT$_4$ receptors was found in the cerebellum (Table 2). Neither the cerebellar cortex nor the dentate nucleus seemed to contain specific [^{125}I]SB 207710 binding sites (results not shown).

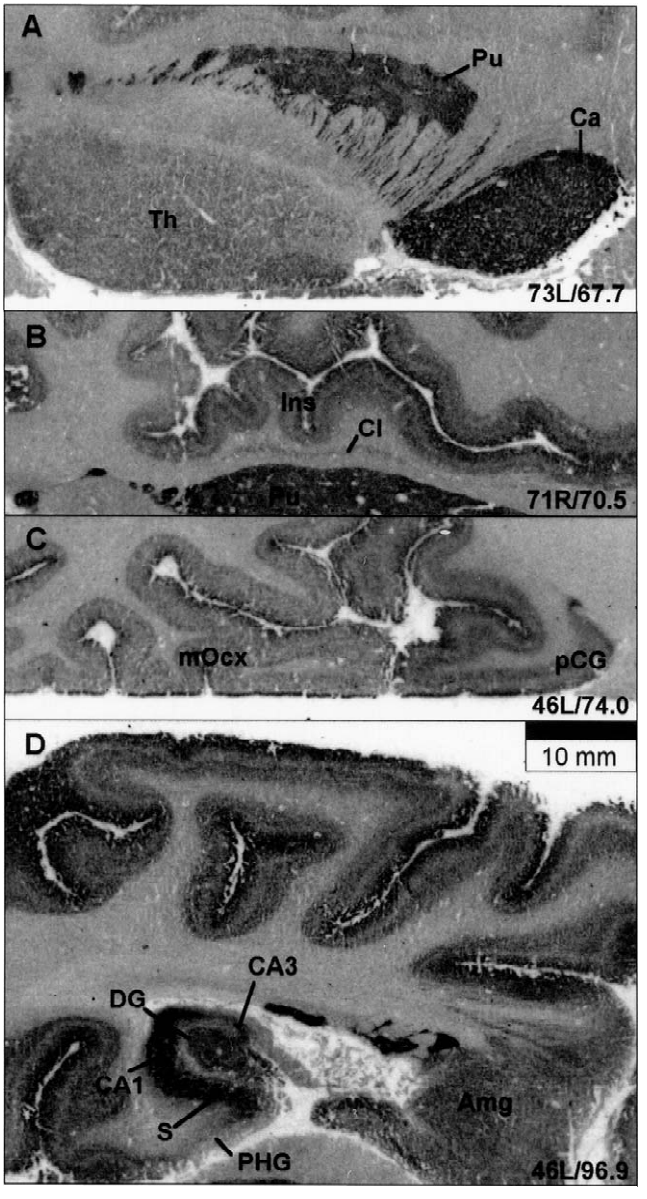

Fig. 3. Details of whole hemisphere autoradiograms showing the total [^{125}I]SB 207710 binding in horizontal sections. (A) Caudate nucleus and putamen, dorsal level. (B) Insular cortex. (C) Medial occipital cortex and posterior cingulate gyrus. (D) Temporal cortex including the hippocampal formation. Abbreviations, see Fig. 1 legend.

4. Discussion

This study shows that [^{125}I]SB 207710 is suitable for the autoradiographic mapping of 5-HT$_4$ receptors in human brain whole hemisphere sections. Studies using [^{125}I]SB 207710 are therefore well suited to provide detailed anatomical correlates for low-resolution SPET images obtained with [^{123}I]SB 207710 in vivo.

The localisation of 5-HT$_4$ receptors in the human brain has been described using a similar autoradiographic methodology and the radioligands [^{3}H]prucalopride and

[^{3}H]R116712 (Bonaventure et al., 2000). However, it could be of importance to evaluate and compare the regional 5-HT$_4$ receptor distribution using other structurally different radioligands. Thus, Jakeman et al. found some regional differences in receptor binding densities in the guinea-pig brain when comparing the distribution of [^{3}H]GR113808 with [^{3}H]BIMU-1 under conditions where the 5-HT$_4$ receptor was labelled (Jakeman et al., 1994). Comparison with previous analyses of 5-HT$_4$ receptors in the human brain postmortem revealed in general similar binding patterns (Bonaventure et al., 2000; Domenech et al., 1994; Reynolds et al., 1995; Waeber et al., 1993). Thus, high levels of [^{125}I]SB 207710 binding were found in the basal ganglia and the hippocampus with lower densities in the neocortex. In contrast to the previous whole hemisphere autoradiographic study in the human brain, where no 5-HT$_4$ binding sites were detected in the thalamus or raphe nuclei (Bonaventure et al., 2000), we observed low but non-negligible specific [^{125}I]SB 207710 binding in these regions. These differences could be due to the higher sensitivity of ^{125}I- compared to ^{3}H-labelled compounds and indicate that [^{125}I]SB 207710 is the preferred radioligand for the detection of 5-HT$_4$ binding sites in low-density regions.

In the hippocampal formation, the highest densities were found in the CA1 region and the subiculum. Our studies in the human brain revealed higher levels in the CA1 than in the CA3 region (30 and 16 pCi/mm^2, respectively), which is in contrast to the guinea-pig brain, where the highest levels were observed in the CA3 region (Jakeman et al., 1994). The reasons for this discrepancy are not clear, although they can be suggested to be due to different radioligands and assay conditions or possible species differences.

Although the 5-HT$_4$ binding densities are markedly lower, the cortical binding pattern of [^{125}I]SB 207710 was generally similar to that obtained for 5-HT$_{1A}$ receptors as found using [^{3}H]WAY-100635 (Hall et al., 1997). [^{125}I]SB 207710 showed high binding densities in superficial layers and a band of diffuse labelling in deep neocortical layers, which is similar to the cortical distribution pattern of the 5-HT$_{1A}$ receptor (Hall et al., 1997). Also in the hippocampal formation, the binding pattern showed some similarities to that obtained using [^{3}H]WAY-100635, with dense binding in the pyramidal layer of CA1 and in the subiculum. This is in line with electrophysiological studies, which have shown that 5-HT$_{1A}$ and 5-HT$_4$ receptors are colocalised on rat CA1 pyramidal cells, where they mediate opposite effects of 5-HT on neuronal excitability (Roychowdhury et al., 1994).

4.1. Conclusion

[^{125}I]SB 207710 can be used for the detailed anatomical description of 5-HT$_4$ receptors in human brain whole hemisphere autoradiography and could provide an important complement to in vivo imaging studies using the recently developed SPET radioligand, [^{123}I]SB 207710. The findings confirm previous reports on the regional localisation of 5-HT$_4$ receptors in the human brain.

Acknowledgements

The excellent technical assistance of Siv Eriksson, David Card, Dr Rachel S. Mulligan and Kerstin Larsson is greatly acknowledged. We are grateful for the discussions on neuroanatomy with Professor Yasmin L. Hurd. This work was supported by the Human Brain Informatics (HUBIN) at Karolinska Institutet and by grants from the Swedish Medical Research Council (MFR) 11640 and the National Institute of Mental Health (NIMH) MH 44814. Parts of the results have been presented in abstract form at the 43rd Meeting of the Scandinavian College of Neuro-Psychopharmacology 10–13 April 2002.

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